Effects of high and low sucrose-containing beverages on blood glucose and hypoglycemic-like symptoms

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ABSTRACT

Background and Aims: There is this intriguing but not yet well-explored suggestion that highly absorbable sucrose-sweetened drinks might exacerbate hunger by promoting temporal hypoglycemia-like responses already in non-diabetic healthy individuals. This might provide a possible additional explanatory mechanism for previous reported associations between consumption of sugar-sweetened drinks and body weight gain. The current study involves two separate and independently conducted human experiments exploring the effects of two different single-doses of sugar-sweetened beverages on temporal blood glucose nadir and possible related behavioral hypoglycemic-like symptoms in healthy participants.

Methods: By way of two separately conducted between-subjects experiments, effects of 1) a low (29g) sugar-containing beverage compared to a sweetened zero-energy drink and a milk drink (experiment-1) or 2) a high (80g) sugar-sweetened beverage compared to a zero-energy and a non-sweetened colored water drink (experiment-2) were measured on changes in blood glucose, behavioral hypoglycemia, appetite and mood.

Results: Experiment-1: The 29g sucrose containing beverage caused a high (37%) glycemic increase and a smaller response (15%) to the milk drink, which both peaked 30min after consumption, whereas the sweetened zero-energy drink had very little effect on blood glucose. Regardless of the different magnitude of peak glycemic responses, both the sugar and milk drinks rather equally caused blood glucose concentrations to return to normal and stable baseline values 90min later. There were no (different) effects of the beverages on behavioral hypoglycemic-like symptoms, appetite or mood. Experiment-2: the 80g sucrose containing beverage caused a large (72%) glycemic peak response at +30min after consumption, whereas neither the sweetened zero-energy nor the non-sweetened colored water drink had any meaningful effect on blood glucose. After intake of the 80g sugar beverage, blood glucose concentrations remained elevated (13%) at +120min and returned to lower baseline values in

the direction of hypoglycemia levels at +165min. There were no (differential) effects of the beverages on behavioral hypoglycemic symptoms, appetite or mood.

Conclusions: The current findings indicate that instead of a low (29g) sugar-containing beverage, a high (80g) sugar-containing beverage caused blood glucose concentrations to fall below baseline values almost reaching hypoglycemia levels at the end of measurements. There were no hypoglycemic-like behavioral symptoms including changes in appetite or mood: at least not at end of measurements +165min after consumption. Since this might include that in particular consumption of high-glycemic index drinks could still promote symptoms in the longer run, further research is needed to explore possible hypoglycemic-like effects of high dosages of sugar-sweetened beverages across more extended/delayed time measurements.

Keywords: Sugar, Blood glucose, Hypoglycemia, Appetite

INTRODUCTION

The worldwide epidemic of overweight and obesity is considered one of the greatest threats to human health. High carbohydrate, high-glycemic index (often sugar-sweetened) foods have been implicated in this epidemic by virtue of undermining appetite control and increasing hunger (1-3).

In line with the classic glucostatic theory of feeding regulation (4), glucose is commonly thought to provide a signal to control food intake; initiating hunger and energy intake when blood glucose concentration is low and/or via cellular gluco-deprivation, and terminating energy intake via satiation when blood glucose concentration rises. Although more research is needed to define what minimal change in plasma glucose is at least needed to affect ingestive behavior (5); normal portions of carbohydrate and/or sugar ingestion are commonly found to increase blood glucose concentration and to decrease hunger and/or short-term food intake (6-8). More precisely, well-conducted controlled studies reveal that sugar consumption (sucrose or its components glucose and fructose) reduces hunger and reduces energy intake within 15-60 minutes and over one or more days (7, 9).

Despite the average findings from the literature, it might however still be possible that highly absorbable sugar in either regular or high portions could cause brief temporal increases in appetite and thereby promote energy intake. Part of the basis of this intriguing assumption is the suggestion that in particular rapidly digestible high-glycemic (GI) carbohydrates and sugars in liquid form may cause an initial rapid increase in blood glucose concentration followed by a rapid surge in insulin release which subsequently pushes blood glucose concentration below a critical (abnormally low or 'hypoglycemic') level, thereby temporarily increasing hunger and energy intake and ultimately promoting weight gain (2, 3). Hypoglycemia' is a severe problem in people with type-1 and type-2 diabetes, mostly due to disturbed insulin-related counter regulation of changes in plasma glucose levels during altered food intake. In people with

diabetes, (severe) hypoglycemic events are often (yet, not always) preceded by episodes of mild antecedent hypoglycemic plasma glucose levels as high as 3.9 mmol/l (10-12).

Hypoglycemia however mostly (almost only) occurs in people with diabetes and thus is hardly found in healthy participants. Still, even in non-diabetic people mild behavioral hypoglycemia-like symptoms may occur; given that glucose counter-regulatory mechanisms may already be triggered at low blood glucose concentrations of approximately 3.6-3.9 mmol/L (13). Although most previous studies on the effects of high-glycemic index foods on blood glucose and/or appetite in first instance may not seem to support the hypoglycemia assumption in healthy non-diabetic participants, most however did not actually test associations between fast temporal (post beverage) insulin-initiated declines in blood glucose and incidences of short-term hypoglycemia-like behavioral symptoms. Most previous studies explored effects of high-glycemic-index foods and/or drinks on the averaged/pooled glucose concentrations (AUC); and hence demonstrated reductions in appetite and/or food intake 1 to 1.5 hours after intake of carbohydrate meals and/or single sucrose portions (14-16). Since in particular consumption of high sugar-containing liquid foods has been associated with body weight gain in children and adults (17-19), this 'rebound' hypothesis might provide a possible additional contributing underlying mechanism.

Accordingly, the current study includes two separate and independently conducted human experiments to test whether either a low or high single-dose of sugar-sweetened drink could already cause post-drink hypoglycemic-like symptoms in healthy non-diabetic individuals. Different groups of eligible healthy participants (n=90 Experiment-1 and n=61 Experiment-2) were monitored for temporal changes in blood glucose, behavioral-glycemic symptoms and appetite before and repeatedly after consumption of a low (29g; Experiment-1) or high (80g; Experiment-2) dose of sugar-sweetened drink as compared to either a milk- and a sweetened

zero-caloric drink (Experiment-1) or a sweetened zero-caloric drink and a non-sweetened colored water drink (Experiment-2). Since sugar consumption has on rare occasions been found to (modestly) alter mood (20-22), measurement of mood was also included in both experiments.

MATERIALS AND METHODS

Participants

Experiment-1

Participants were recruited from a large database of n=700 undergraduate students at Maastricht University. All participants received an invitation mail to complete an on-line survey set on the UM digital research platform 'EMIUM'. The survey included a questionnaire screening package concerning general information (health, personal or family history of medical or psychiatric complaints, smoking and drinking habits, caffeine consumption, weight and height, use of psychoactive drugs) and several questionnaires concerning relevant symptoms, psychopathology and (abnormal/deviant or irregular) eating styles. The screening package ended with a question whether students were willing to participate in a laboratory study focusing on the influence of drinking different beverages on behavior. They were invited to participate if they 1) were able to join during the summer (May-June 2016) and 2) if they reported absence of chronic or current physical or psychiatric illness; family history of psychiatric illness; medication use; metabolic, hormonal or intestinal diseases; irregular diets; excessive use of alcohol (>2 units a day), coffee (>10 units a day), cigarettes or other drugs; aversion for certain foods; and pregnancy. Following this selection, n=90 participants were finally included in the study. Participants were between 17-26 years of age (mean age= 23±1.8). The study was carried out in accordance with the Helsinki Declaration of 1975 as revised in 1983 and approved by the Ethical Committee of the Faculty of Psychology and Neurosciences. All participants gave their informed consent and were paid for participation.

Experiment-2

Participants were recruited from undergraduate Psychology students at Maastricht University; by way of local university advertisement (posters, flyers) and social media**.** They were asked to participate and to sign up at the UM digital student research-participating platform 'SONA'. On SONA, they received global information about the study ('focusing on the influence of drinking different beverages on behavior') and were invited to participate if they: 1) were able to join in May-June 2019 and 2) if they reported absence of chronic or current physical or psychiatric illness; family history of psychiatric illness; medication use; metabolic, hormonal or intestinal diseases; irregular diets or deviant eating habits; excessive use of alcohol (>2 units a day), coffee (>10 units a day), cigarettes or other drugs; aversion for certain foods; and pregnancy. Eligible students then scheduled themselves for their study session and received a confirmation mail. Eventually, n=61 student-participants were included in the study. Participants were between 18-28 years of age (mean age= 21.4±2). The study was carried out in accordance with the Helsinki Declaration of 1975 as revised in 1983 and approved by the Ethical Committee of the Faculty of Psychology and Neurosciences. All participants gave their written informed consent and received 5 ECTS study participation credits (part of the Bachelor program) and a free lunch-coupon for participation.

Design and Procedure

Experiment 1 and 2

In order to prevent carry-over effects that have been found to occur in dietary studies using within-subjects designs (16), both experiments were conducted according to a placebo-controlled, between-subjects design. Both experiments were conducted in the same season (May-June) and comparable laboratory settings, yet in different years (2016 and 2019). In both

experiments, participants were assigned to one of three treatment conditions and visited the laboratory for monitoring of their mood, appetite, blood glucose concentrations and behavioural hypoglycaemic symptoms before and repeatedly after consumption of either; 1) a 250-ml beverage containing sugar (29g sucrose), milk (Milk) or zero-calorie sweetener (Experiment-1) or 2) a 250-ml beverage containing sugar (80g sucrose), zero-calorie sweeter, or non-sweet coloured water (Experiment-2).

In both experiments, participants were instructed to refrain from alcohol for at least 36 hours and to fast 12 hours before onset of the test sessions; only water or caffeine-free tea without sugar was permitted. Participants were told that a compliance check would be conducted directly at arrival via a first glucose measure. Figure 1 shows a schematic diagram and time table of the experimental procedure. INSERT FIGURE 1 ABOUT HERE

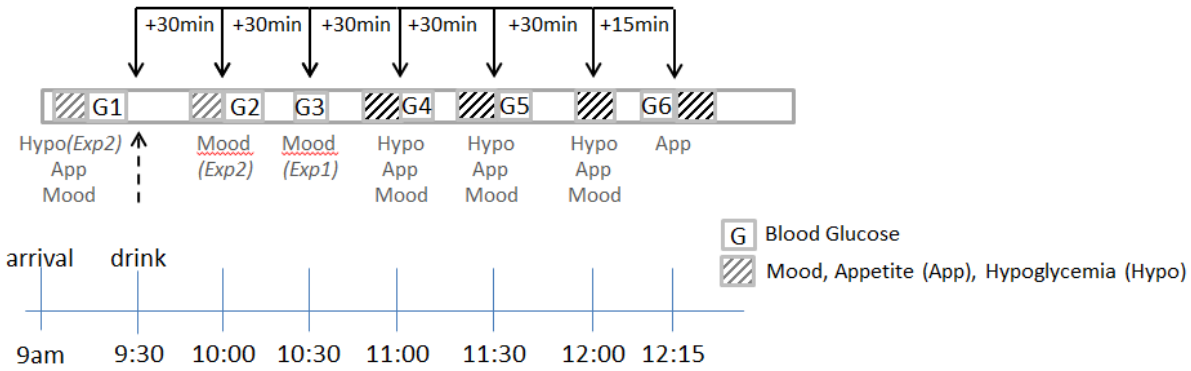

Fig 1: Schematic diagram and time table of the experimental procedure used in **Experiment-1** and **Experiment-2**.

During each laboratory midweek test day, a maximum of 6 (Experiment-1) or 4 (Experiment-2) participants arrived at the laboratory waiting room at approximately 9am. Ten minutes after arrival and a little rest, participants received plenary instructions and then were directed into their private test compartment (as part of a large experimental room) to conduct a sequence of repeated tests and blood glucose measurements before and after beverage intake.

After receiving instructions, a pre-drink baseline measurement of mood, appetite and behavioral hypoglycemia was conducted (and an extra brief questionnaire/measurement for another study) followed by a first blood glucose sample. Then participants consumed their beverage within approximately 2 minutes after which five subsequent blood glucose measures were taken every +30min (the final/last measure was taken at +165min). Before and repeatedly after beverage intake mood, appetite and hypoglycemia symptoms were assessed on frequent occasions. In between blood sampling, for approximately 25min, participants stayed in their private test compartment and were permitted to rest and/or to read magazines (see Figure 1 for detailed overview of the time-test protocol). [At the end of the study, participants also conducted a brief questionnaire/attention measure for another study]. All measurements, tests and rest periods in both experiments were supervised by test assistants (trained masters-students) who were blind to the drink conditions.

Beverages

Experiment-1

Three different 250-ml beverages were included comprising a sugar-drink (29g glucose/fructose; 119 kcal), a semi-skimmed milk drink (118 kcal) or a sweetened zero-energy placebo (PLC) beverage (5 kcal). The Sugar and PLC beverages were manufactured and tested to reach equal flavour, appearance and sweetness (Knowledge-center Sugar and Food, Baarn, The Netherlands & United Softdrinks, Utrecht, The Netherlands). For each test day, beverages were prepared (weighed, labeled and stored in the refrigerator) one day before being served by a research assistant who supervised consumption of the preload within the 2min allowed (see Table 1 for nutrient constitution). The research assistants were blind to the drinks served (they couldn’t see the differences in appearance between the milk and the two sweet drinks), as were

the participants for the two sweet drinks. Before each test day, prepared beverages were (re)labeled D1, D2 and D3 (order was shuffled for each new week) and allocated to the subjects' identification numbers by a staff member not involved in the daily measurements and/or in contact with the participants. INSERT TABLE 1 ABOUT HERE

Table 1: Composition of the 250ml Sugar^{29g}, sweet Placebo (PLC) and Milk beverage used in **Experiment-1**

	Sugar^{29g}	**PLC**	**Milk**
Nutrient (g)			
Sucrose	29	-	11.3
Fruit juice concentrate	0.2	1	
Sweet modulator flavor	*	*	
Water (ml)	225	245	
Protein			8,8
Fat			3
Kcal	119	5	118

**aspartame, acesulfame-K, raspberry aroma*

Experiment-2

Three different 250ml beverages were included comprising a sugar-sweetened drink (80g sucrose; 336 kcal), a sweetened zero-energy PLC beverage (no sugar: 18 kcal) and a non-sweetened colored water drink (Control).

The Sugar and zero-energy PLC beverages were tested to approach equal flavour, appearance and sweetness (including a small taste-pilot at the university). The non-sweetened colored water (control) drink was tested for equal (color) appearance. For each test day, beverages were prepared (weighed, labeled and stored in the refrigerator) one day before being served by trained student-research assistants (interns) who supervised consumption of the preload within

the 2min allowed (see **Table 2** for nutrient constitution). The research assistants were blind to the drinks served, as were the participants for the two sweet drinks. Before each test day, prepared beverages were labeled A, B or C and allocated to the subjects' identification numbers by a staff member not involved in the daily measurements and/or in contact with the participants. INSERT TABLE 2 ABOUT HERE

Table 2: Composition of the 250ml Sugar^{80g}, sweet Placebo (PLC) and non-sweet colored water (Control) beverages used in **Experiment-2**

	Sugar^{80g}	**PLC**	**Control**
Nutrient (g)			
Sucrose	80	-	-
Fruit juice concentrate	20	18	
Sweet modulator flavor	*	*	-
Water (ml)	100	100	250
Colorant (ml)			0.7
Kcal	336	18	0

**acesulfame-K, sucralose, steviol glycoside, raspberry aroma*

Measurements

Experiment 1 & 2

Mood

Positive and negative affect were measured by using the Positive And Negative Affect Scale-questionnaire (PANAS (23). This questionnaire contains 10 items for positive affect (e.g. interested, excited, proud) and 10 items for negative affect (e.g. nervous, upset, irritable). Total scores for both scales are a sum of the scores on their respected items which all range from 1 (not at all) to 5 (extremely).

In Experiment-1, mood measures were taken at baseline (pre-beverage) and 60, 90, 120 and 150 minutes after beverage intake. In Experiment-2, mood was measured at baseline (pre-beverage) and 30, 90, 120 and 150 minutes after beverage intake.

Appetite

Appetite was measured using 100mm visual analogue scales (VAS) to assess; 1) Hunger, 2) Fullness, 3) Desire to eat a meal and 4) Desire to eat a snack. These (or comparable) questions are rather commonly used with VAS scale in appetite research (24). For each item, scores ranged from 0mm (not at all) to 100mm (Very much). An average (total) Appetite score was calculated at each time point by the formula: Appetite = [desire to eat a meal + desire to eat a snack + hunger + (100 - fullness)] / 4.

In Experiment-1, appetite was measured at baseline (pre-beverage) and 60, 90, 120 and 165 minutes after beverage intake. In Experiment-2, appetite was measured at baseline (pre-beverage) and 90, 120, 150 and 165 minutes after beverage intake.

Hypoglycemia symptoms

Because postprandial hypoglycemia signs (including changes in appetite) are most likely expected within 60-120min after food intake, behavioral manifestation of hypoglycemia was assessed for either (between); 60-90, 90-120 and 120-150 minutes after beverage intake (Experiment-1) or before (0) and 60-90, 90-120 and 120-150 minutes after beverage intake (Experiment-2). Behavioral hypoglycemia signs were assessed by probing for the following symptoms; 1) dizziness/lightheadedness, 2) heart palpitations, 3) tingling fingers or feet, 4) shakiness, 5) heavy sweating, 6) sudden chills and/or heavy cold and 7) sudden urges to eat**.** For each item, participants were asked whether they experienced it during the last 30min (at

+90min, +120min and +150min after beverage intake). Each item was scored by "0" (No) or "1" (Yes); with higher sum scores indicating more hypoglycemia symptoms.

Blood Glucose

For both separate experiments, blood glucose was measured at baseline (pre-beverage) and 30, 60, 90, 120 and 165 minutes after beverage intake by a finger-prick procedure, using Accu-Chek FastClix Sterile 0.3mm lancet devices. Each time, one drop of blood was placed on a one-touch test strip for immediate readings of glucose concentration (mmol/L) with the Accu-Chek Aviva monitor device (Roche GmbH, Mannheim, Germany). Participants remained seated during blood samplings.

Statistical analyses

Data were first examined for accuracy of data-entry and missing or extreme values. The main research questions were analyzed by means of repeated measures multi- or univariate analyses of variance ([M]ANOVA) by using the General Linear Model (GLM: SPSS 15.0 for Windows) with *Treatment* (Experiment-1= Sugar^{29g} vs. Milk vs. PLC; Experiment-2= Sugar^{80g} vs PLC vs Control) as between-subjects factor and *Time* (Before vs several time measures after beverage intake) as within-subjects factors for the several dependent measures (blood glucose, appetite, hypoglycemia symptoms, mood). Given the focus of the study, exploring hypoglycemia signs related to time-dependent post-peak glucose changes, pre- and repeated post-measurements of blood glucose concentrations were included as repeated factors (instead of a pooled AUC value).

Only significant main or interaction effects revealed by these procedures were further examined by univariate pairwise-comparison tests across testing times for each treatment condition separately. In the initial analyses, gender was included (separately) as covariate; although

excluded from the final analyses due to its insignificance. Huynh-Feldt or Greenhouse-Geisser corrected P values are reported when sphericity assumptions were not met. All statistics are evaluated at a significance level of 5% (two-tailed). Data is reported as means ± SD (text) or as means ± SEM (figures).

RESULTS

Blood Glucose changes

Experiment 1

Repeated measures multivariate analysis of variance with *Treatment* (Sugar^{29g} vs. Milk vs. PLC) as between-subjects factor and *Time* (Before^{T0} vs. After $^{+30, +60, +90, +120 \text{ and } +165}$ beverage) as within-subjects factors on blood glucose revealed a main significant effect of *Time* [$F(5,86)=32,423$; $P<0.0001$] and a significant interaction of *Treatment* x *Time* [$F(10,174)=6,965$; $P<0.0001$]; indicating that blood glucose changes significantly differed between treatment conditions. Pairwise comparisons for time-related blood glucose changes per treatment condition revealed, as shown in Figure 2(A), that blood glucose peaked 30min after the Sugar^{29g} ($P<0.001$) and Milk ($P<0.001$) drink as compared to a clear lack of response following the sweet PLC drink ($P>0.4$); and both raised glucose levels returned to their baseline values +90min after intake (P values >0.5). Sugar^{29g} produced a greater increase in blood glucose (37%: from 4.9±0.4 to 6.7±1.1) than did Milk (15%: from 4.8±0.5 to 5.5±0.8) ($P<0.0001$). After Sugar^{29g} intake, blood glucose concentrations declined somewhat to below baseline values at +120min (4.7±0.6; $p=0.038$) and +165min (4.6±0.5; $p=0.03$), whereas for PLC this was only found at +120min (4.5±0.6; $p=0.029$) and did not happen after milk consumption ($p=0.1$ and $p=0.9$). There were no significant differences in baseline blood glucose levels between Sugar^{29g} (4.9mmol/l), Milk (4.8mmol/l) and PLC (4.8mmol/l) treatments ($P>0.18$). After Sugar29g intake, there were 5 incidences in which blood glucose dropped below 3.9mmol/L; at +90min (3.6 mmol/l), +120min (3.4 and 3.6 mmol/l) and +165min (3.6 and 3.8 mmol/l). For PLC, there were 10 incidences; at +60min (3.7 and 3x 3.8 mmol/l), +90min (3.5 mmol/l), +120min (3.2, 3.3 and 3.6 mmol/l) and +165min (3.4 and 3.8 mmol/l). After Milk, there were 3 incidences; +90min (3.6 and 3.7 mmol/l) and +165min (3.6 mmol/l).

INSERT FIGURE 2 ABOUT HERE

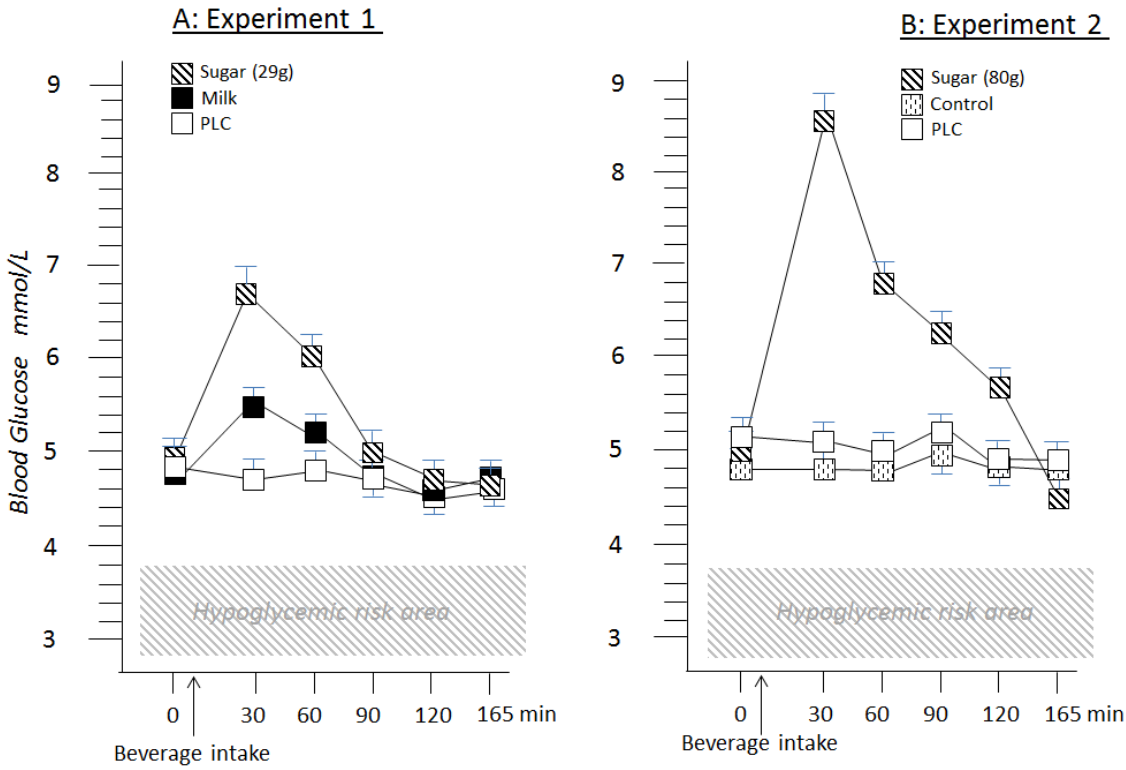

Fig 2: Changes in blood glucose concentrations across time. Experiment-1: after a low (29g) sugar drink, a milk drink and a Placebo drink (PLC). Experiment 2: after a high (80g) sugar drink, a colored water drink (control) and Placebo (PLC).

Experiment 2

Repeated measures multivariate analysis of variance with *Treatment* ($Sugar^{80g}$ vs. PLC vs. Control) as between-subjects factor and *Time* ($Before^{T0}$ vs. After $^{+30, +60, +90, +120 \text{ and } +165}$ beverage) as within-subjects factors on blood glucose revealed a main significant effect of *Time* [$F(5,54)=31,152$; $P<0.0001$] and a significant interaction of *Treatment* x *Time* [$F(10,110)=9,207$; $P<0.0001$]: indicating that blood glucose changes significantly differed between treatment conditions. Further pairwise comparisons revealed (see Fig 2B) that blood glucose concentration significantly increased only after $Sugar^{80g}$ consumption and peaked at a 72% increase at +30min ($P<0.001$) as compared to a clear lack of response after the sweet PLC drink (all p-values >0.2) and Control drink (all p-values >0.6). After $Sugar^{80g}$ intake, blood glucose concentrations remained elevated at +90min ($p<0.001$) and +120min ($p=0.019$) and

declined to below baseline values (4.4±0.7: p<0.001) at the end of the measurements +165min after intake. Blood glucose concentrations also declined somewhat below baseline values after PLC intake at +120min (4.9±0.4: p<0.001) and +165min (4.9±0.5: p=0.008). There were no significant differences in baseline blood glucose levels between Sugar80g (5±0.56), PLC (5.2±0.42) and Control (4.8±0.32) treatments (P>0.08). Additional analysis revealed only 4 incidences in which blood glucose levels dropped below 3.9mmol/L; exclusively after Sugar80g intake at +120min (3.7mmol/l) and at +165min (3.1, 3.6 and 3.8 mmol/l).

Behavioral Hypoglycemia symptoms

Experiment 1

Analysis of variance with *Treatment* (Sugar^{29g} vs. PLC vs Milk) as between-subjects factor and *Time* (+60-90, +90-120, +120-150) on the total number of behavioral hypoglycemia symptom did not reveal any significant effect; no effect of *Time* (P>0.4) and no effect of *Time* by *Treatment* (P>0.6). Table 3 shows the incidence of behavioral symptoms across time between treatment conditions. INSERT TABLE 3 ABOUT HERE

Experiment1: Incidence of behavioral symptoms of hypoglycemia across treatment and time

	TREATMENT			
Minutes after intake	**Sugar^{29g}** [N=29]	**PLC** [N=30]	**Milk** [N=31]	*P value*
+ 60-90	5 (17%)	7 (23%)	5 (16%)	➡ NS (0.49)
+ 90-120	4 (14%)	5 (17%)	4 (13%)	➡ NS (0.43)
+ 120-150	6 (21%)	5 (17%)	4 (13%)	➡ NS (0.43)
P value	⬇ NS (0.53)	⬇ NS (0.49)	⬇ NS (0.65)	

Table 3: Number of participants reported at least one symptom +60-90; +90-120 and + 120-150 after intake of treatment beverage (Sugar^{29g}, sweet PLC or Milk). Incidence of hypoglycemic responses were assessed by dizziness; tingling; heavy sweating, cold or shaking and heart palpitations.

An additional comparison was conducted to see whether behavioral hypoglycemic symptoms were reported by participants with the lowest (<3.9mmol/l) blood glucose concentrations (see previous results). In the Sugar^{29g} condition there were 5 incidences (3.4-3.6 mmol/l) without complaints. In the PLC condition there were 11 incidences (3.2-3.8mmol/l) with 3 participants reporting complaints (from early start of measurements onwards); and in the Milk condition there were 3 incidences (3.6-3.7mmol/l) with 2 participants reporting complaints.

Experiment 2

Analysis of variance with *Treatment* (Sugar^{80g} vs. PLC vs Control) as between-subjects factor and *Time* (Before T0, +60-90, +90-120, +120-150) on the total number of behavioral hypoglycemia symptom did not reveal any significant effect; no effect of *Time* (P>0.07) and no effect of *Time* by *Treatment* (P>0.7). Table 4 shows the incidence of behavioral symptoms across time between treatment conditions. INSERT TABLE 4 ABOUT HERE

Experiment 2: Incidence of behavioral symptoms of hypoglycemia across treatment and time

	TREATMENT			
Minutes after intake	**Sugar^{80g}** [N=22]	**PLC** [N=18]	**Control** [N=21]	*P value*
T0 (pre-drink)	2 (9%)	5 (28%)	6 (29%)	➡ NS (0.50)
+ 60-90	2 (9%)	2 (11%)	4 (19%)	➡ NS (0.55)
+ 90-120	1 (5%)	1 (6%)	0 (0%)	➡ NS (0.65)
+ 120-150	2 (9%)	3 (17%)	4 (19%)	➡ NS (0.60)
	⬇	⬇	⬇	
P value	NS (0.60)	NS (0.27)	NS (0.38)	

Table 4: Number of participants reported at least one symptom before (T0) and +60-90; +90-120 and + 120-150 after intake of the sugar beverage (Sugar^{80g}), sweet PLC beverage (no sugar) or the Control beverage (colored water). Incidence of hypoglycemic responses were assessed by dizziness; tingling; heavy sweating, cold or shaking and heart palpitations.

An additional comparison was conducted to explore whether behavioral hypoglycemic symptoms were particularly (more profoundly) reported by the 4 participants with the lowest

(<3.9mmol/l) blood glucose concentrations (see previous results). None of these participants reported any symptoms.

Appetite

Experiment 1

Repeated measures analysis of variance with *Treatment* (Sugar^{29g} vs. PLC vs. Milk) as between-subjects factor and *Time* (Before^{T0} vs. After $^{+60, +90, +120 \text{ and } +165}$ beverage) as within-subjects factors on the composite appetite scores (0-100mm) only revealed a main significant effect of *Time* [$F(4,84)=50,797$; $P<0.0001$]; indicating that feelings of appetite changed as a function of time regardless of treatment condition. As shown in Figure 3(a), appetite significantly increased across time after sugar^{29g} intake as well as after milk intake and after PLC intake. There were no significant differences between beverages; as indicated by the absence of a significant *Treatment* x *Time* interaction ($P>0.4$).

Additional correlational analyses were conducted on relationships between blood glucose changes and appetite from baseline to 90min and 165min after intake. These analyses did not reveal any significant relationships between blood glucose and appetite changes across time and no differences were found with respect to drink condition (all $r <0.18$; all $p >0.3$).

INSERT FIGURE 3 ABOUT HERE

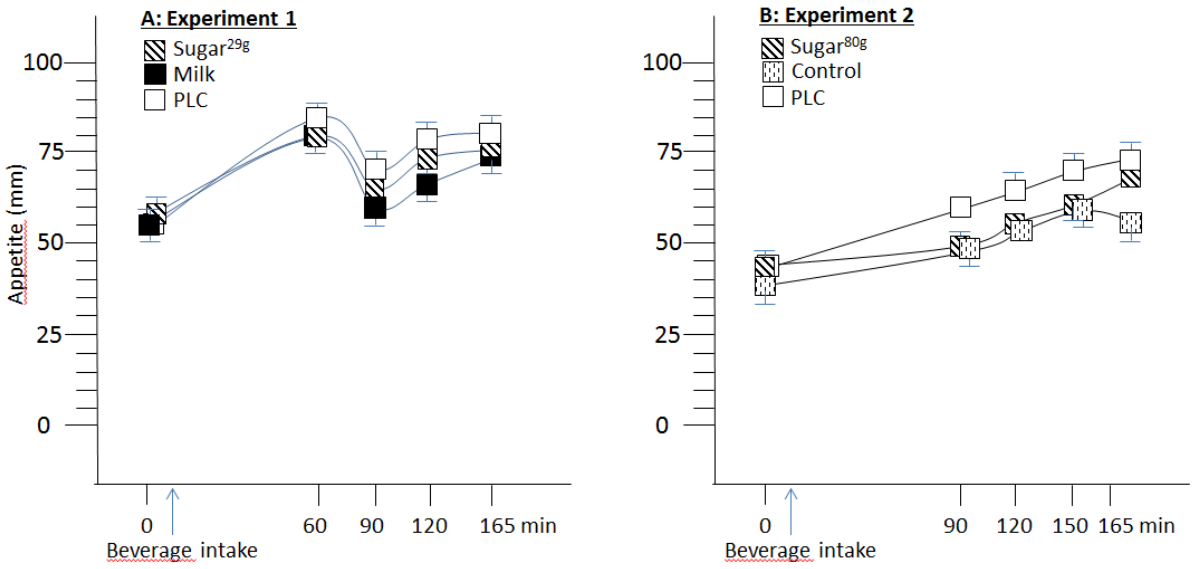

Fig 3: Appetite changes across time. Experiment 1: after a low (29g) sugar drink, a milk drink and Placebo (PLC). Experiment 2: after a high (80g) sugar drink, a colored water drink (control) and a Placebo drink (PLC).

Experiment 2

Repeated measures analysis of variance with *Treatment* (Sugar^{80g} vs. PLC vs Control) as between-subjects factor and *Time* (Before^{T0} vs. After $^{+90,\ +120,\ +150\ \text{and}\ +165}$ beverage) as within-subjects factors on the composite appetite scores (0-100mm) only revealed a main significant effect of *Time* [$F(4,55)=23{,}546$; $P<0.0001$]; indicating that feelings of appetite changed as a function of time regardless of treatment condition. As shown in Figure 3(b), appetite significantly increased across time after sugar intake as well as after sweet PLC intake and after Control intake. There were no significant differences between beverages; as indicated by the absence of a significant *Treatment* x *Time* interaction ($P>0.1$).

Additional correlational analyses were conducted on relationships between blood glucose changes and appetite from baseline to 90min and 165min after intake. These analyses did not reveal any significant relationships between blood glucose and appetite changes across time and no differences were found with respect to drink condition (all $r<0.1$; all $p>0.2$).

Mood changes

Experiment 1

Repeated measures analyses of variance with *Treatment* (Sugar^{29g} vs. Milk vs PLC) as between-subjects factor and *Time* (Before^{T0} vs. After $^{+60,\ +120,\ +150\ \text{and}\ +165}$) as within-subjects factors on changes in Negative and Positive mood both revealed violations of the sphericity assumption (Mauchly: P<0.001). Subsequent Greenhouse-Geisser corrected analysis on negative mood did not reveal any significant main (P>0.08) or interaction (P>0.19) effect, whereas greenhouse-geisser corrected analysis on positive mood only revealed a main significant effect of *Time* [F(4,84)=31,068; P<0.0001]; indicating a modest reduction in positive mood across time (23.5±6 > 21.7±7 > 20.9±7 > 18.4±7 > 18.1±7) regardless of treatment condition.

Experiment 2

Repeated measures analyses of variance with *Treatment* (Sugar^{80g} vs. PLC vs Control) as between-subjects factor and *Time* (Before^{T0} vs. After $^{+30,\ +90,\ +120\ \text{and}\ +165}$) as within-subjects factors on changes in Negative and Positive mood both revealed violations of the sphericity assumption (Mauchly: P<0.001). Subsequent Greenhouse-Geisser corrected analysis only revealed a main significant effect of *Time* both on negative mood [F(4,50)=16,93; P<0.001] as well as on positive mood {F(4,54)=29,0]; indicating a modest reduction in negative mood (12.5±3 > 11.7±2 > 10.8±1 > 10.5±0.9 > 10.7±1) as well as in positive mood (21.9±5.3 > 19.8±6 > 18±5.2 > 16.6±4.6 > 16.1±4.7) across time, regardless of treatment condition.

DISCUSSION

By way of two separate independently conducted human experiments, effect of a low (29g) or high (80g) sugar-sweetened beverage was explored on possible occurrence of serological as well as behavioral signs for hypoglycemia within a 30-165min post-absorption interval. The sugar drinks revealed large dosage-dependent increases in blood glucose responses that were not seen after the non-energetic sweet placebo drinks. The rise in blood glucose concentrations was much greater after 80g compared to 29g sugar beverage intake and only after the 80g beverage blood glucose declined below baseline values (in the direction of hypoglycemic levels) at the end of measurements (+165min). There were no beverage-specific changes in the incidence of hypoglycemia-like behavioral symptoms.

Effects of a low and high sugar containing drink on blood glucose changes

As expected, peak blood glucose concentrations rapidly increased after the low 29g sugar beverage (34%) and this effect was even doubled after consumption of the high 80g sugar beverage (72%) as compared to milk (17%) and the sweetened zero-energy drinks (no change). Peak blood glucose responses for both the low and high sugar containing beverages were equally found at 30min post beverage intake. After consumption of the low (29g) sugar containing beverage, blood glucose returned (and remained) at normal fasting baseline values (>4.5mmol/L) from +90- to +165min. After consumption of the high (80g) sugar containing beverage, blood glucose concentrations remained elevated by 13% at +120min and returned below baseline values (4.4mmol/L instead of 5mmol/L baseline) at the end of the measurements at +165min.

Since antecedent hypoglycaemia in diabetes patients is commonly defined when blood glucose values drop below approximately <4mmol/L (10, 25), this in first instance seems to indicate

that neither consumption of a low (29g) or high (80g) glycemic sugar beverage is likely to cause hypoglycaemia-signs, and therefore may not be expected by this route either to alter (quickly increases) appetite or to trigger sympathetic or neurogenic symptoms characteristic of hypoglycaemia. That means: at least not until +165min after consumption in this sample of healthy volunteers tested under laboratory conditions. Hence, blood glucose concentrations after the high (80g) sugar-containing drink did show a more delayed return-to-baseline fall (still 13% increase at +120min) than found after the low (29g) sugar drink (steady return from +90min onwards) and even returned below baseline values in the direction of hypoglycaemic levels at the end of measurements (up to +165min). We thus do not know whether blood glucose values might have dropped even below critical hypoglycaemia values following the end of measurements (after +165min onwards). This does not seem to be unrealistic. In a previous study, the consumption of a tonic drink containing 60g of sugar already led to a blood glucose nadir in the hypoglycaemic range (3.8 mmol/L) at +195min (without behavioural or hormonal hypoglycaemia symptoms (26)). It thus would be worthwhile exploring further the effects of high doses of sugar drinks over more prolonged post-consumption times on possible hypoglycaemic events in healthy non-diabetic individuals.

Partly based on previous discoveries of gut sweet taste receptors, it has sometimes been speculated that sweet taste alone (including non-energetic sweeteners) may affect glucose control and hence glycemic responsiveness (27). This however could not be supported by previous in vivo studies in humans or animals (28, 29) and was also not supported by a recent randomized controlled trial that investigated the effect of sucralose consumption three times per day for 12 weeks on glucose homeostasis in normal healthy volunteers (30). Consistent with this, the current results revealed no marked effect of non-energetic sweeteners (aspartame, acesulfame-K or sucralose) on glycemia.

Behavioural hypoglycemic symptoms; physiological signs, appetite and mood

In line with the absence of a post-prandial hypoglycemic rebound effect on blood glucose concentrations, the current findings did not reveal any significant effect of the low or high sugar drink condition on behavioral changes indicative of hypoglycemia up to the final measurement, namely +150min after beverage intake.

-Physiological signs

Most commonly reported behavioral symptoms indicative of reactive hypoglycemia are dizziness, tingling, heavy sweating, cold or shaking or heart palpitations; and these occurred very rarely (31). There were however no such changes in physiological hypoglycemic symptoms from 30-150min after beverage intake; regardless of beverage condition and regardless of its high or low sugar content.

-Appetite

In line with the average absence of postprandial hypoglycemic changes; the current results did not reveal a beverage-related change in subjective appetite. In both separate and independent experiments, there was only a modest general increase in appetite over time regardless of drink condition. This increase in appetite is to be expected as participants were fasted overnight and the test sessions in both experiments ended shortly before lunchtime.

It has sometimes been assumed that when calories are consumed in a high absorbable liquid form, the body's appetite control system might miss it; bypassing satiation and hence increasing appetite/food intake (32). The current lack of a difference in the effects on appetite between the

calorie-containing drinks (sugar and milk) and the control-drinks (zero-energy drinks or the water drinks) seems to support this assumption (the differences in nutrient content went undetected since all the treatments were given in liquid form). This also seems to fit previous findings of sugar-containing liquid foods contributing more to weight gain than solid foods (17, 18). Nevertheless, many previous studies used procedures in which participants consumed a drink containing sugar (e.g. glucose, sucrose or fructose), or a control drink (water, or a drink sweetened with a low-energy sweetener), and measured appetite and/or food intake. Overall, these studies did show a reduction in food (energy) intake after consuming sugar (sucrose, glucose or fructose), regardless of whether it was consumed in a liquid or solid vehicle (6-9, 16). Studies have revealed reductions in appetite and/or related eating behavior within 30-60min after sugar drinks, with more prolonged satiety for larger amounts (>130g) of sucrose (7, 33) and hence for larger higher glycemic responses (15, 34). In a more recent meta-analysis however, semisolid or solid preloads were nevertheless found to lead to larger subsequent energy compensation than did liquid preloads (35). In addition, alterations in energy intake after high glycemic preload consumption may even appear in absence of changes in rated appetite (36), suggesting a relative insensitivity of appetite ratings as direct predictors for changes in eating behaviour. So even in the absence of current beverage-related temporal changes (increases) in appetite ratings, it still remains most realistic to prevent too much energy intake from sugar-containing liquid foods to reduce the risk for weight gain in children as well as in adults (see also (17-19).

-*Mood*

The current findings on mood revealed no treatment- (beverage-) specific changes in mood. In both experiments, there were only modest reductions in positive feelings over time regardless

of the drink condition. In Experiment 2, this modest reduction in positive mood was also accompanied by a very modest (but significant) reduction in negative mood; which is an unexpected result and seems counterintuitive.

A few previous placebo-controlled studies have suggested that carbohydrate drinks might improve mood either due to sweet taste reward or due to changes (increases) in plasma tryptophan availability for uptake into the brain (20, 22, 37). Yet, common findings on the effects of sugar/carbohydrates on mood are rather mixed and a recent meta-analysis challenged the idea that acute sugar or carbohydrates intake could meaningfully improve mood, at least in healthy participants (38). In addition, only few experiments revealed very small mood improvements after carbohydrate intake exclusively under stressful circumstances and/or in vulnerable participants (37, 38). Overall, current findings comply with the averaged findings that sucrose intake does not have an effect on mood changes in normal circumstances and/or in healthy participants.

Limitations

A first limitation is that appetite was not also (firstly) measured +15min after beverage intake. In the current studies, appetite measurements started +60min (Exp-1) or +90min (Exp-2) after beverage intake; due to the expected timing of an insulin-related drop in blood glucose concentration following beverage intake (approximately between +60-120min). However, since previous studies have reported reductions in appetite already +15 minutes after intake (as a likely direct effect of increased blood glucose), it remains unclear whether this also might have happened in the current studies. A second limitation might be the absence of controlling for possible pain experience caused by finger-prick procedure. Although participant did not verbally indicate experiences of pain (asked/monitored during the studies), some of them might

still have experienced physical discomfort that could have partly influenced mood and appetite across time.

Conclusion

The current findings suggest that consumption of a low (29g) or high (80g) sugar-sweetened beverage is not likely to cause serological and/or behavioral hypoglycemia symptoms in healthy non-diabetic participants within 30-165min after consumption. However, since the high (80g) sugar-containing beverage actually did cause blood glucose to fall below baseline values at the end of measurements (in the direction of hypoglycemic values); further research is needed including extended assessments of possible hypoglycemic events across different high doses (or types) of sugar drinks.

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